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Research Article

COMPARATIVE EVALUATION OF ANTI-PYRETIC AND ANALGESIC ACTIVITIES OF *RUNGIA REPENS* NEES AND *RUNGIA PECTINATA* LINN.

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ABSTRACT

The objective of the present study was to evaluate the anti-pyretic and analgesic activity of hydroalcoholic extract (50:50) of *Rungia repens* Nees and *Rungia pectinata* Linn. The anti-pyretic activity of both the plant was studied separately in Brewer's yeast-induced pyrexia in rats. The analgesic activity of both the plant was studied separately using acetic acid- induced writhing and tail immersion method in mice. Both the plant extract at a dose level of 400 and 800 mg/kg body weight showed significant activity. The average antipyresis recorded after 5 h of treatment with hydroalcoholic extract of *R. repens* were 0.89^{0}C and 1.55^{0}C at the dose levels of 400 and 800 mg/kg body weight respectively. Similarly, antipyresis for *R. pectinata* was also recorded as 1.36^{0}C and 1.71^{0}C at 400 and 800 mg/kg body weight dose levels respectively. Both fthe extracts (400 and 800 mg/kg body weight) produced analgesic activity in dose dependent manner but in comparisons the hydroalcoholic extracts of *R. repens* shown better analgesic activity as compared to *R. pectinata*. Whereas the anti-pyretic activity of *R. pectinata* is better as compared to *R. repens*.

Key words: *Rungia repens*, *Rungia pectinata*, analgesic, anti-pyretic.

INTRODUCTION

The species belonging *Rungia* have been claimed to exhibit anti-pyretic, diuretic, and antifungal properties. The whole plant of *R. repens* has been used throughout the ages for the treatment of fever and cough, and is also considered as vermifuge. Fresh, bruised leaves are mixed with castor oil applied to scalp to cure tinea capitis, a scaly fungoid infection, usually occurring among children etc[1-3]. Juice of the small and somewhat fleshy leaves of *R. pectinata* another *Rungia* species is considered cooling and aperient and is prescribed for children suffering from small pox in dose of a table spoonful or two twice daily. The bruised leaves are applied to contusions to relieve pain and diminish swelling. Among the Santals, the root is given as a medicine in fevers[4].

MATERIALS AND METHODS

Plant material

Rungia repens and *Rungia pectinata* plants were collected from nearby areas of Salipur, Orissa and identified at Botanical Survey of India, Howrah. Their voucher specimen was deposited in the Institute herbarium.

Preparation of extracts

The shade dried aerial parts of *R. repens* and *R. pectinata* were powdered in a pulverizer separately. The powdered drug was extracted with Ethanol water mixture (50:50) for 72 h in Soxhlet apparatus. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was distilled followed by evaporation in a rotary flash evaporator. The concentrated extracts were transferred to pre-weighed flasks. After evaporation of the residual solvents the weight of the each extract was recorded. The yields were 8.52 and 7.64 % w/w for *R. repens* and *R. pectinata* respectively. Phytochemical screening gave positive tests for carbohydrates, amino acids, fixed oils, phytosterols, glycosides, tannins and phenolic compounds. Interestingly both the plant contains similar type of phytoconstituents when subjected to preliminary phytochemical screening.

Experimental animals

Wistar rats of either sex weighing 150-200 g were used for screening of anti-pyretic activity. Swiss albino mice of either sex weighing 20-25 g were used for screening analgesic activity. Animals were housed in groups of six per cage at a temperature of 25 ± 1^{0}C and relative humidity of 70 ± 5%. A 12:12 hour light: dark cycle was followed during the experiments. Animals had free access to food and water, however, food was withdrawn six hours before and during the experiments. The animals were obtained from the Central Animal House of Institute of Pharmacy & Technology, Salipur. The experimental protocol was approved by the Institutional Ethical Committee (1053/ac/07/CPCSEA). The hydroalcoholic extract of both the plants were devoid of any mortality or behavioral changes when the animals were given upto 800 mg^{-1} p.o in rats and mice.

Anti-pyretic activity

The method described by Evangelista *et al*. was used[5]. Rats of either sex were divided into six groups, comprising six in each group. Yeast induced pyrexia was used to evaluate the anti-pyretic activity of the test compounds. The body temperature of each rat was recorded by measuring the rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) following a standard method. The rats were allowed to remain quiet in the cage for some time.

A thermister probe was inserted 3-4 cm deep into the rectum after fastening the tail to record the basal rectal temperature. The animals were then given a subcutaneous injection of 10 ml/kg of 15%w/v Brewer's yeast suspended in normal saline and the animals were returned to their housing cages. Nineteen hours after yeast injection, the rats were again restrained in individual cages to record their rectal temper1ature. Immediately the test compounds and standard were administered orally at their respective doses. Rectal temperature of all the rats was recorded at 19 h immediately before the administration of test compounds, vehicle and paracetamol (150 mg/kg.) and again at 1 h intervals up to 5 h after the administration. The differences between the actual values and the starting values were registered for each time interval. The maximum reduction in rectal temperature in comparison to the control group was calculated. The results were compared with the effect of standard drug paracetamol 150 mg/kg p.o.

Acetic acid induced writhing test

The hydroalcoholic extracts were evaluated for its analgesic activity by acetic acid-induced writhing model[6, 7]. Swiss albino mice were divided into six groups of six animals each. First group was used as negative control and received distilled water (5 ml/kg body weight), an hour before injection of 0.6 % v/v acetic acid (10 ml/kg) intraperitonially.

Second group served as positive control and received aspirin 200 mg/kg body weight p.o. The third and fourth group received the extracts of *R. repens* at dose levels of 400 mg/ kg and 800 mg/kg body weight respectively. Similarly fifth and sixth group received the extracts of *R. pectinata* at the same dose level an hour before acetic

acid injection. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administration of acetic acid[8]. Percent protection against writhing movement was taken as index of analgesia.

Table 1: Effect of *R. repens* and *R. pectinata* on yeast induced pyrexia

Treatment	Dose mg/kg	Yeast Induced Pyrexia Temperature in ^{0} C					
		0 h	1 h	2 h	3 h	4 h	5 h
Control	0.5% w/v CMC solution	37.71 ± 0.12	37.68 ±0.14	37.42± 0.16	37.58± 0.20	37.44± 0.23	37.54± 0.24
Paracetamol	150	37.16 ± 0.09	36.64 ± 0.21*	36.34± 0.11*	35.59± 0.08**	35.14± 0.14**	35.10± 0.17**
R. repens	400	37.26 ± 0.15	36.87± 0.11*	36.62± 0.07*	36.56± 0.11**	36.48± 0.10*	36.37± 0.24*
	800	37.65 ± 0.07	37.48± 0.09*	37.01± 0.18**	36.54± 0.19**	36.32± 0.13**	36.10± 0.13**
R. pectinata	400	37.14 ± 0.16	36.90± 0.13*	36.78± 0.10*	36.24± 0.09**	35.62± 0.15*	35.78± 0.11
	800	37.12 ± 0.12	36.72± 0.14*	36.63± 0.10**	36.31± 0.11**	36.08± 0.11**	35.41± 0.18**

n=6, Values are mean ± SEM ***P*<0.01(significant),**P*<0.05(significant) values are compared with control group

Table 3: Effect of *R. repens* and *R. pectinata* on tail immersion response in mice

Group	Drug dose mg/kg,i.p.	Predrug (mean±SEM) reaction time (in sec)	Reaction time in sec (mean + SEM) 30 min	1h	2h	4h
Distilled H_2O	1ml/kg	3.5±0.4	4.3±0.49	4.0±0.5	4.3±0.5	4.2±0.4
R.repens	400	3.2±0.6	6.1±0.6	7.5±0.5*	7.0±0.4*	7.3±0.3*
R.repens	800	3.0±0.5	8.0±0.73*	7.8±0.6*	8.2±0.5*	8.8±0.3*
R. pectinata	400	2.7±0.3	5.0±0.51	6.2±0.4*	5.8±0.5	6.2±0.6*
R. pectinata	800	2.8±0.4	5.3±0.61	5.8±0.7*	5.2±0.6	5.6±0.6*
Pethidine	5	3.7±0.3	9.0±0.73*	9.3±0.6*	9.2±0.3*	8.2±0.4*

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. *P <0.001* vs Control (n= 6)

Tail immersion method

The method described by Siegmund *et al.* was used[6]. Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided into six groups of six animals each. The animals of first group was treated with distilled water and served as control. The group treated with pethidine (5 mg/kg; p.o.) served as positive control[9]. The third and fourth group received the extract of *R. repens* at dose levels of 400 mg/ kg and 800 mg/kg body weight respectively. Similarly fifth and sixth group received the extract of *R. pectinata* at the same dose level. About 5 cm of the tail of mice was dipped in warm water, kept constant at 55 ± 0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 15 sec. The latent period of the tail immersion response was taken as the index on analgesic and was determined immediately after injection.

Statistical analysis

The statistical analysis was carried out with SPSS 10.0 (Windows) software. Difference of the parametric data of body temperature(s) was examined by two way analysis of variance (ANOVA) with Dunett's Post hoc pair wise multiple comparison t test, to compare a set of experimental data against control mean.

RESULTS

The average antipyresis recorded after 5 h of treatment with hydroalcoholic extract of *R. repens* were 0.89^{0}C and 1.55^{0}C at the dose levels of 400 and 800 mg/kg body weight respectively. Similarly, antipyresis for *R. pectinata* was also recorded as 1.36^{0}C and 1.71^{0}C at 400 and 800 mg/kg body weight dose levels respectively. The standard drug paacetamol at 150 mg/kg, p.o body weight produced significant decrease in elevated body temperature. This study suggests that antipyresis produced by both the plants' extract is directly proportional to the administered dose (Table 1 & Fig. 1). The results were found to be highly significant (*P*<0.05) in comparison to the control. The hydroalcoholic extracts of *R. repens* and *R. pectinata* (400 and 800 mg/kg, *p.o*) significantly suppressed the acetic acid-induced writhings in a dose-dependent manner. The standard drug aspirin at 200 mg/kg body weight produced significant inhibition of writhing movements (Table 2 & Fig. 2). The results were found to be highly significant (*P<0.001*) in comparison to the control. The number of writhing movements during 30 min of observation in the control group was 80.17±2.48 which corresponds with the findings of other workers[10, 11].

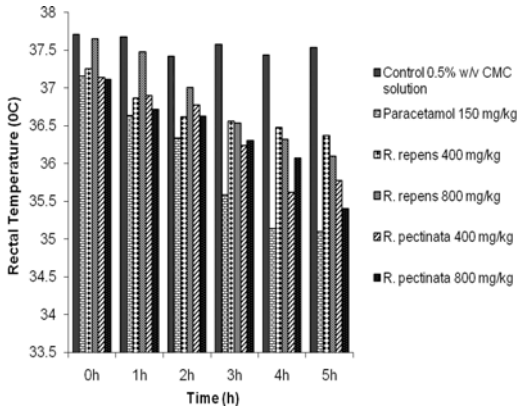

Fig. 1: Anti-pyretic activity of *R. repens* and *R. pectinata*

The hydroalcoholic extracts of *R. repens* and *R. pectinata* (400 and 800 mg/kg, *p.o*) significantly suppressed the acetic acid-induced writhings in a dose-dependent manner. The standard drug aspirin at 200 mg/kg body weight produced significant inhibition of writhing

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Table 2: Effect of *R. repens* and *R. pectinata* on acetic acid induced writhing response in mice

Group	Acetic acid induced writhing response in mice Drugdose mg/kg,s.c	No. of writhing movements	% Protection
Normal saline	8ml/kg	80.17±2.48	-
R.repens	400	38.83±1.77*	51.56
R.repens	800	27.33±1.02*	65.91
R. pectinata	400	50.50±0.99*	37.01
R. pectinata	800	43.00±1.78*	46.36
Aspirin	100	16.67±0.88*	79.21

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. *P <0.001* vs Control (n= 6).

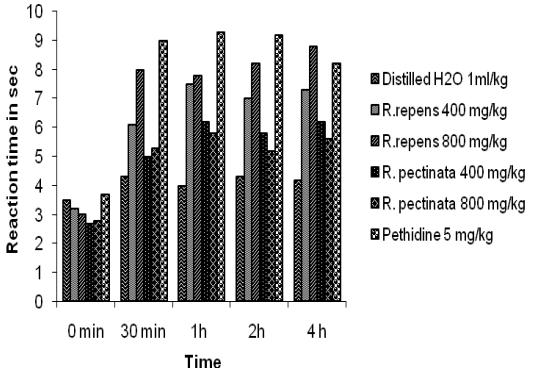

Fig. 2: Effect of *R. repens* and *R. pectinata* on acetic acid induced writhing response in mice

In the tail immersion model, both the extracts (400 and 800 mg/kg body weight) produced analgesic activity in dose dependent manner but in comparision the hydroalcoholic extract of *R. repens* shown better analgesic activity as compared to *R. pectinata* (Table 3 & Fig. 3). This justifies its use in traditional system of medicine.

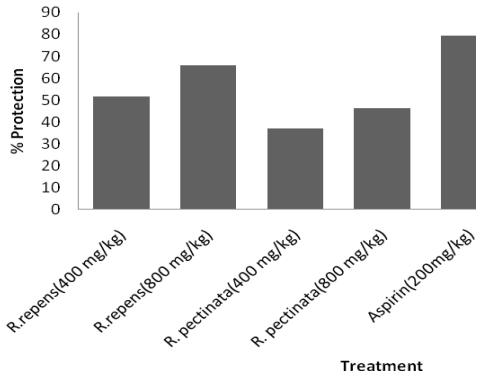

Fig. 3: Effect of *R. repens* and *R. pectinata* on tail immersion response in mice

The results showed that the hydroalcoholic extract of *R. repens* and *R. pectinata* possess a significant anti-pyretic and analgesic effect in maintaining normal body temperature and in reducing algesic effect in rats and mice respectively in a dose dependent manner and its effects are comparable to that of the standard drug. Overall, studies confirmed the ethnomedicinal claims.

Moreover, the statistical analysis with two-way ANOVA showed that the hydroalcoholic extract of *R. repens* and *R. pectinata* decreases yeast induced elevated body temperature, thermal and acetic acid induced algesic model in a dose dependent manner as compared with control group. The results were found to be highly significant (*P<0.001*) in comparison to the control.

DISCUSSION

Fever may be due to infection or one of the sequele of tissue damage, inflammation, graft rejection, or other disease states. Anti-pyretic are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.

In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature[12]. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[13]. Elevated body temperature and pain are two major signs of the body against inflammation[14].

A drug with anti-inflammatory activity usually exhibit anti-pyretic and analgesic properties[15]. The best examples would be the nonsteroidal anti-inflammtory drugs, which possess all three activities[16]. Previously, it was reported that *R. repens* and *R. pectinata* extract has diuretic, anti-inflammatory activity in rats and antimicrobial properties against a number of pathogenic microorganisms[17].

The writhing response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is considered to involve local peritoneal receptors. The number of writhing movements during 30 min of observation in the control group was 80.17±2.48 which corresponds with the findings of other workers[10, 11].

In the tail immersion model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center.

In the tail immersion model, there was no significant difference in the mean predrug reaction time between the different groups. Thirty minutes after drug administration, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. The test drug produced a dose-dependent increase in the reaction time at various time intervals of observation.

The mechanism underlying the activity of *R. repens* and *R. pectinata* in anti-pyretic and analgesic is still unknown. Preliminary phytochemical screening of the hydroalcoholic extract of both the plant species gave positive test for carbohydrates, amino acids, fixed oils, phytosterols, glycosides, tannins and phenolic compounds, which might be partly responsible for the anti-pyretic and analgesic activity reported in the current investigation.

Therefore, the overall results obtained suggested that the hydroalcoholic extract of *R. repens* and *R. pectinata* might relieve pain, provide some justification for the folklore use in the treatment of fever and pain. Further studies are going on to isolate the bioactive principles responsible for anti-pyretic and analgesic activities with their mechanism of action.

CONCLUSION

The hydroalcoholic extracts of *R. repens* and *R. pectinata* ariel part exhibits anti-pyretic and analgesic activity at a dose level of 400 and 800 mg/kg body weight. In comparison antipyretic activity of *R.*

pectinata is better than *R. repens* at the same dose level. Whereas, the hydroalcoholic extract of *R. repens* shown better analgesic activity as compared to *R. pectinata*. This finding provides some scientific evidence on the traditional use of both plants.

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